The Sporangiospore Germination *in vitro*, in *Osmunda japonica*

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Abstract

Pinnules of sporophylles in Osmunda japonica were cultured stationarily at the bottom of the culture solution. After about a month, prothalia were crowded out through the walls of sporangia which appeared to be dead. Then, prothalia multiplied and grew up, and they formed eventually a cushionlike mass. At five or six months after the inoculation, sporophytes of the next generation appeared on these cushionlike masses of protalia.

These phenomena suggest that the particular genetic information maintained in *Osmunda* may be expressed. It may be similar to the genetic information contained in the first land plants, and they might express it in order to protect reproductive organs against the flood damage. This behavior, namely the sporangiospore germination in the Paleozoic land plants might be effective to protect reproductive organs against both the flood damage and the drought damage. For this reason, the ancient land plants might evolve toward the modern seed plants.
Key words : *Osmunda*, sporangiospore germination, land plant, evolution.

Introduction

In ancient times from the beginning of life, all organisms lived in the water. In the Silurian period, some of plants advanced to lands. They evolved into modern land plants after many and long periods. Many books concerning with these processes have been published (Bold 1987, Gifford and Foster 1987, Nishida 1972). The first organisms might be initiated in sea water or diluted sea water, and then they might evolve in the sea. Following evolution, they might get to land probably not directly from sea to shore, but perhaps from sea water to fresh water and then landing. At the time when some of plants succeeded in landing at first, any other organisms never existed on the land. There existed neither fertile soil nor shade of trees. The rain water got muddy and ran harshly on the bare ground, and the watercourses were unstable. In the places where water was stagnant, namely ponds, marshes and lakes, the water sank rapidly following a short drought, and dried up. And then, on the next rainy day, they became promptly full of water, and overflowed. A flood turned to a drought, and a drought turned to a flood alternately. Even if they were modern land plants, they could not live safely in such severe environments.

Plants grown in shallow places of ponds, marshes or lakes might wither and die during a drought. Plants grown at deep regions lived principally underwater lives. Plants grown in the middle zones lived ocasionally underwater lives or land lives depending upon the variable water levels. They were amphibious plants. Some of them were just the first landing plants.

The Silurian land plants are not yet described in details. The considerable sum of knowledge about the Devonian land plants are provided (Gensel and Andrews 1984). The Devonian land plants might develop sporangia generally on the upper or highest parts of the plants. Not only land life plants, but also water life plants projected their spolangia into the upper air. If sporangia of the Paleozoic land plants were held usually in the air, it was necessary to protect the sporangia against the flood damage. For this purpose, the sporangia did not dehisce, spores were not release, and sporangiospores germinated, in a certain group of land plants. Some of this group, I assume, evolved into seed ferns subsequently. In this way, the sporangiospore germination resulted in the xerophilous adaptation. Thus, land plants advanced from the waterside to the arid area, and eventually, I assume, evolved into the seed plants.

This evolution are resulted by the continuous and sequential acquirement of genetic informations concerning with this evolution. The modern land plants, which are undoubtedly descended from the Paleozoic land plants, may maintain such genetic informations which were effective in ancient land plants. If so, I assume, in sporangia of modern pteridophytes which are placed at the bottom of culture solution, sporangiospores may germinate, and prothallia may be formed, and then gamates may be produced on the prothallia, and the fertilization may occur and result in zygotes, and then the zygotes may develop eventually sporophytes. Based on the hypothesis above-mentioned, this investigation was attempted and carried out.

Materials and Methods

Materials used were *Osmunda japonica.* this species is extensively distributed in the temperate east Asia, and belongs to Osmundaceae. This family is contained in Filicales or Osumundales (Makino 1989, Tagawa 1959,Yamagishi 1974).

Osumunda sporophylls used in culture were collected in March and April among the forest in the vicinity of our university. Immediately after the collection, each pinnule, which bore closed sporangia, was excised. The pinnules were washed with running water during 30 minutes. And then, they were dipped into 70% ethyl alcohol in a moment, and after that, they were sterilized by soaking in 10% bleaching powder solution during 30 minutes. The pinnules were subsequently washed with sterilized water in an aseptic manipulation box. And finally, each pinnule was inoculated into each Erlenmeyer flask which contained the culture solution and cotton plugged. They were cultured under continuous illumination at 25℃. The pinnules were submerged into the culture solutin, and laid on the bottom of the flasks. They were cultured stationarily during long term and observed frequently. The culture solution used in this investigation was the modified White's solution (Choshi 1979). It consisted of 360mg $MgSO_4$, 200mg Na_2SO_4, 200mg $Ca(NO_3)_2$, 80mg KNO_3, 65mg KCl, 16.5mg NaH_2PO_4, 4.5mg $MnSO_4$, 1.5mg H_3BO_3, 1.5mg $ZnSO_4$, 0.75mg KI, 8mg Fe-citrate, 20g saccharose, 1g yeast extract, and 1000ml distilled water. The culture solution was autoclaved

Results

Immediately after the inoculation, the pinnules of *Osmunda* sporophyll were vivid green as a whole, that is, each sporangium was vivid green. From several days after the inoculation, they turned to brownish gradually, that is, dull green sporangia and brown sporangia were mixed on every pinnule. Further more days after, whole pinnules became dark brown, and therefore they appeared dead.

In spite of the appearance, about one month after the inoculation, those sporangia were tinged with greenish colour, and suddenly, green thallus-like plantlets sprouted and burst through the walls of sporangia. Green eruptions were throughout each pinnule (Fig. 1). The facts were revealed by observations with a dissecting microscope, that this phenomenon was not the released spore germination following the sporangium dehiscence, and that it was the sporangiospore germination without sporangium dehiscence, and that prothallia, *i. e.* gametophytes, developed within each sporangium, and grew and exceeded easily the capacity of each sporangium, and then the sporangia were burst, and many prothallia sprouted through the wall of sporangia (Fig. 2).

When the culture was maintained stationarily, these prothallia grew thick, and formed together a cushion-like mass per pinnule (Fig. 3). When the cultures were continued, these prothallia masses enlarged gradually in size, whereas the culture solution decreased gradually in volume. Therefore the upper part of the prothallia mass protruded into the air from the surface of the culture solution.

Each culture was continued further more. At five or six months after the inoculation, many plantlets, which were probably sporophytes, appeared on the outside of the cushion-like mass of prothallia (Fig. 4). It was probable that antheridia and archegonia might be formed on the prothallia, and sperms and egg cells might be produced, and sperms might swim in the culture solution and then fertilize, and produced zygotes might develop into sporophytes. Although each important point is not yet confirmed, this presumption may prove right.

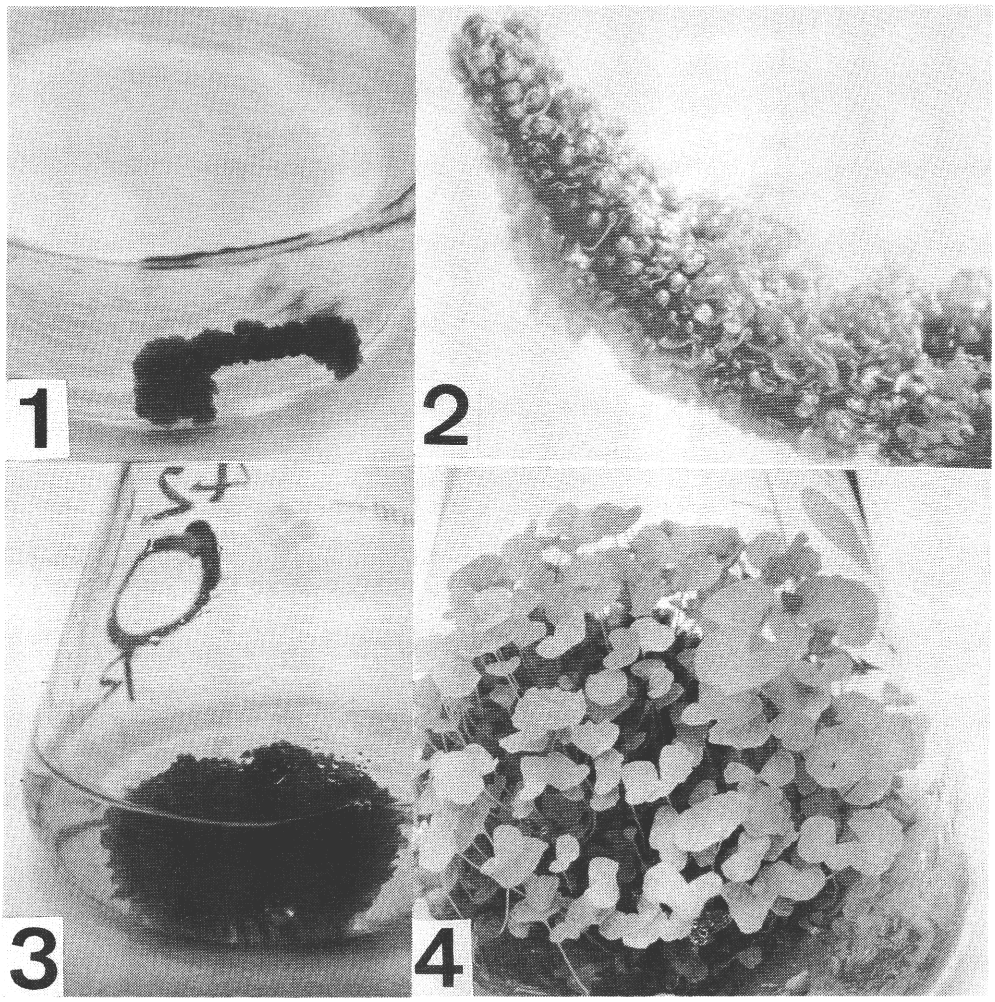

Fig. 1 A pinnule of *Osmunda* sporophyll cultured in the nutritious solution. Gametophytes have sprouted up.

Fig. 2 Microscopic observation of *Osmunda* gametophytes which have sprouted through the wall of sporangia.

Fig. 3 A cushion-like mass of *Osmunda* gametophytes.

Fig. 4 *Osmunda* sporophytes which have appeared on the outside of a cushion-like mass of gametophyts.

In these experiments, that is, the stationary culture of sporophyll pinnules of *Osmunda* at the bottom of culture solution, the materials could be obtained during only forty days in March and April. For this reason, these experiments were repeated for five years. The above-mentioned results were reproduced in every year. Unseasonable weather in early spring caused the contamination of various germs. In each year, the above-mentioned processes and results were surely reproduced in the uncontaminated flasks.

Discussion

In some species of pteridophytes, sporangiospores germinated in sporangia, and so gametophytes were formed in sporangia, and then gametes were produced in sporangia, and fertilization was accomplished in sporangia, and as the result, embryos of sporophytes were generated in sporangia, and these sporangia were protected with several envelopes, and these pteridophytes were consequently evolved into spermatophytes, that is seed plants. This conception above-mentioned is generally accepted (Bold *et al.* 1987, Gifford and Foster 1987, Nishida 1972). Therefore, the sporangiospore germination is regarded as the first step to the evolutional process from the pteridophyte to the spermatophyte. The above-mentioned results suggest that the genetic informations, which were concerned with the ancestral experiences in the evolutional process and which are still now maintained in some species of pteridophytes, may be expressed in *Osmunda* by submergence into the culture solution.

It is generally accepted that the land plant evolution has been directed toward the xerophilous adaptation, especially the closed envelope formation around gametophytes, gametes, zygotes, and embryos. This interpretation appears to be proved by wakes of plant evolution. Nevertheless, it must be reconsidered whether the initial reason of such evolutional direction was the increase of xerophilous ability or not. At the early stage of terrestrial plant life, the pioneer plants were surrounded by severe circumstances. Early land plants which were subjected to a serious drought were certainly killed. On the contrary, eary land plants which were surrounded by indulgent water circumstances, that is at the waterside, probably survived. For such waterside land plants, it was one of unavoidable threats that the plant body with reproductive organs was submerged by swollen waters after rainfalls. Against such a threat, land plants might evolve envelopes surrounding reproductive organs.

The protoplasm which constitutes the organism is jelly-like, and resembles to jam and marmalade. Now, let two bottles of jam are here. If one bottle is uncapped and exposed to direct sunlight, the jam will be soon dried up. If the other bottle is uncapped and put into the water, the jam will absorb water and soon become swollen. How is the jam rescued ? The correct answer is that both bottles must be capped. If the capped bottles are placed under the direct sunlight or into the water, the jam will be protected against the harmful environments. Thus, a closed vessel generates the resistance against both desiccation and submergence. Even though the resistance against desiccation was obtained as a result, it is possible that the initial motivation was the resistance against submergence. I see great possibilities in this idea. I call above-mentioned conception "a bottle of jam hypothesis".

By the way, there exist actually modern living pteridophytes that their sporangiospores are germinated within sporangia and that their gametophytes are formed within sporangia. They are pteridophytes belonged in *Hydropteridales* and some other pteridophytes. Pteridophytes belonged in *Hydropteridales*, as the name implies, grow in watery environment and their sporangia are submerged. The *Hydropteridales* is generally regarded as peculiar pteridophytes. Nevertheless, above-mentioned results suggest that *Osmunda* may have similar genetic informations to those in *Hydropteridales*. The reconsideration of the evolutional significance in *Hydropteridales* may be required.

There may exist the criticism that above-mentioned results are not natural but artificial. Certainly I have not yet observed the sporangiospore germination of *Osmunda*. But nevertheless, based on the above-mentioned results, it is possible that *Osmunda* sporophylls are pushed down and submerged by muddy water flow during a heavy rain, and then that their sporangiospores are germinated within their own submerged sporangia. It is probable that suchlike phenomena happen somewhere on the present earth.

there may also exist another criticism that the facts described in this article are not scientific experiments but horticulture within flasks. That's right. But, this article is the first one of a series of articles which will be published in the near future. In this article, the newly discovered facts are described as they are. The description of facts is the root of science and the first step to science. The scientific experiments are expected to be described in the next and subsequent articles.

References

Bold, H. C., C. J. Alexopoulos and T. Delevoryas (1987) Morphology of plants and fungi, fifth edition. Harper and Row Publishers, New York.

Choshi, A. (1979) Effects of IAA on the flowering of excised plumules in *Pharbitis nil*. *Rep. Fac. Sci., Shizuoka Univ.* **13**, 97-104.

Gencel, P. G. and H. N. Andrews (1984) Plant life in Devonian. Praeger Publishers, New York.

Gifford, E. M. and A.S. Foster (1987) Morphology and evolution of vascular plants, third edition. W. H. Freeman and Co., New York.

Makino, T. (1989) Revised Makino's new illustrated flora of Japan (in Japanese). Hokuryukan, Tokyo.

Nishida, M. (1972) Tane no oitachi (which means "Origin of seed" in Japanese). Iwanamishoten, Tokyo.

Tagawa, M. (1959) Coloured illustrations of the Japanese pteridophyta (in Japanese).

Hoikusha, Osaka.
Yamagishi, T., ed. (1974) Fundamentals of phylogeny in plants (in Japanese). Hokuryukan, Tokyo.